them under the electron microscope. The observations were realized with a Philips 300 EM[9].

Results and discussion. In the fine sections we observed the binucleate cells subjected to the action of the 3'AdR for 3, 5, 7 and 9 h (Figure 1). The presence of 3'AdR for 2 h before the caffeine treatment justifies us in assuming that the binucleate cells produced subsequently have gone through their anaphase and telophase under the effect of the 3'AdR.

The nuclei of these cells show numerous lobulations at the surface. The prenucleolar material can be observed in the interchromatinic spaces in the form of small round bodies of varying sizes (Figure 2b and c) and of argyrophilic nature (Figure 2 a). These prenucleolar bodies appear compact and consist of apparently fibrillar material, homogeneously distributed and possessing an electron density similar to that of the pars fibrosa of a normal nucleolus. In the first few hours of the interphase, the prenucleolar bodies generally do not show any vacuoles inside them, but in the course of the following hours, in addition to some small bodies, other larger bodies appear with vacuoles in them. Both the smaller and the larger ones generally show granules 400–500 Å in diameter on the periphery and on the inner surface of the vacuoles (Figure 2g). In some of the prenucleolar bodies observed, there is a small 'cap' of material that appears to be trabecular in nature.

With the uranyl-EDTA-lead method, which is selective for RNA staining[7], the prenucleolar bodies show up in strong contrast, while the chromatin remains unstained (Figure 2e). With this staining technique, the granules as well as the described 'caps' appear positive (Figure 2f–g).

With silver impregnation, the prenucleolar bodies stand out clearly in the nucleus (Figure 2d), appearing homogeneous in point of staining intensity and size of silver grains, which are generally smaller than those seen in the rest of the nucleus.

As morphological elements typical of telophase nuclei, the prenucleolar bodies have already been studied in previous papers[1,2], and it is worth calling attention to their argyrophilia[10,11] and to their staining affinity with uranyl-EDTA-lead[9,12]. The absence of nucleolar reorganization in the telophase, while the prenucleolar bodies are still present under the effect of RNA synthesis inhibitors, has been studied under the light microscope[1,2]. This paper confirms the results previously obtained, and the staining peculiarities of the prenucleolar material. The phenomenon of vacuolization observed in the larger prenucleolar bodies – probably due to the coalescence of smaller bodies – appears to be similar to that which is found in interphase nucleoli treated with 3'AdR[2]. In addition to the prenucleolar material of fibrillar character, and in close connexion with it, we can also observe other ultrastructural elements: the small caps of apparently trabecular material, similar to the 'coiled bodies'[13], and granules measuring 400–500 Å in diameter which seem to resemble those observed in *Allium cepa* interphase nucleoli under the action of 3'AdR[14].

Resumen. El estudio ultraestructural de los cuerpos prenucleolares en células meristematicas de *Allium cepa*, en las que se ha inhibido la reorganizacion del nucleolo por accion del 3'AdR, revela la naturaleza fibrilar de los mismos, la existencia sobre ellos de granulos de 400–500 Å de diámetro, así como formaciones a modo de «cap» de material con aspecto trabecular. Se observa a lo largo del tratamiento un fenómeno de vacuolación semejante al descrito en nucleolos interfasicos tratados con 3'AdR.

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Neural Differentiation in Aggregates Containing Mixtures of Cell Types

One of the most striking aspects of neural development is the differentiation of several types of neurons which associate themselves by means of specialized junctions, the synapses. Very little is known about the cell interactions required for the development of the synaptic junction. The great complexity of the embryonic neural tube makes it very difficult to analyse these interactions in vivo, so that it is advisable to use simplified models. Reaggregation cultures[1,2] which have proved to be important tools for studying cell interactions, have been used to study various aspects of neural differentiation[3–9]. This culture technique is being used in our laboratory to study interactions between different types of neural cells or between neural and non-neural cells when they become associated in 'combined' aggregates. Some of the ultrastructural observations reported here indicate that this procedure can give valuable information on the mechanisms which regulate synapse formation.

Optic lobe (OL), telencephalon (T), neural retina (NR) and limb (LB) cells from 7-day-old White Leghorn chick embryos were used. These tissues were chosen because some of them (e.g.: neural retina and optic lobe) are known to interconnect selectively during development. Before dissociation, OL and T were freed from surrounding mesenchyme, and NR isolated from pigmented retina. Dissociation was carried out as previously described[4,5], following with some modifications the procedures developed by MOSCONA[1] and STEINBERG[2]. Cell suspensions were adjusted to a final concentration of 10–20 ×

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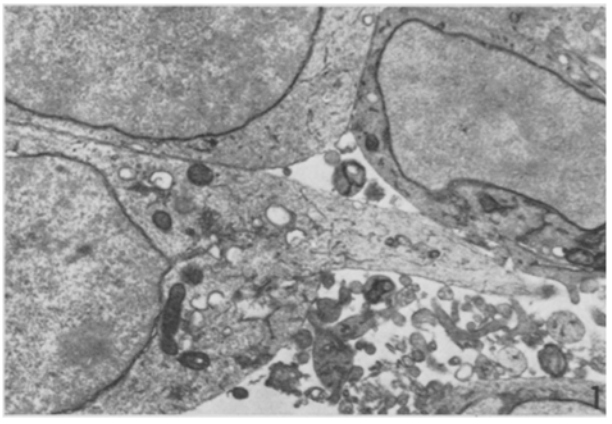

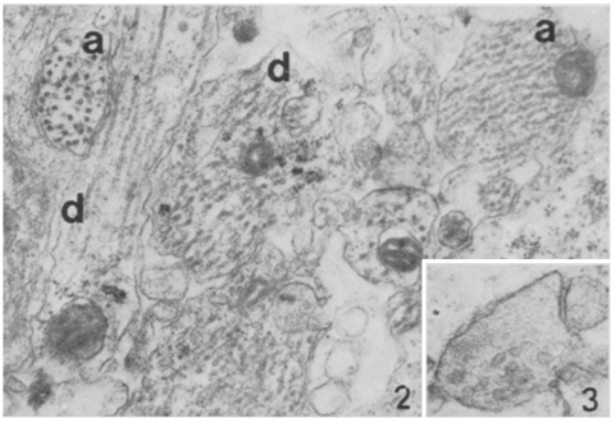

Fig. 1. Aggregate formed after 8 days in culture by optic lobe (OL) cells. Several neuroblasts and numerous cell processes can be seen. Postfixation in potassium permanganate. ×8,800.

Fig. 2. Same type of aggregate as in Figure 1, but postfixed in osmium tetroxide. 'Axon-like' processes (a) contain microtubules and mitochondria, 'dendrite-like' processes (d) contain ribosomes as well. ×20,000.

Fig. 3. Detail of a cell process belonging to the same material as in Figure 1. A cluster of clear vesicles of 400–600 A can be seen within the process. ×32,000.

10^6 cells/ml in culture medium (medium 199 supplemented with 10% chicken serum and 10% 9-day-old chick embryo extract). In the 'pure' cultures, 3 ml aliquots of one of the cell suspensions were placed in 25 ml Erlenmeyer flasks sealed with rubber stoppers. In the 'combined' cultures, 1.5 ml aliquots of two of the cell suspensions were mixed before culturing. The flasks were placed in a water bath at 37 °C with gyratory agitation at 90 rpm. The culture medium was changed twice weekly. After 8 days in culture, the resulting aggregates were fixed for 1 h in the cold with a mixture of equal volumes of culture medium and 5% glutaraldehyde in 0.1 *M* cacodylate buffer, pH 7.4, followed by a brief washing in the same buffer. They were further processed for 1 h with either 1% osmium tetroxide in 0.1 *M* cacodylate buffer, or 0.8% potassium permanganate in the same buffer, dehydrated in acetone and embedded in Epon 812.

'Pure' aggregates. After 8 days in culture, OL aggregates show many neuroblasts (Figure 1). The cell somas have a lobulated nucleus, and their cytoplasm is rich in ribosomes. Numerous cell processes (Figure 1), of 2 types (Figure 2), are observed. 'Axon-like' processes contain mitochondria and longitudinally oriented microtubules, whereas 'dendrite-like' processes contain ribosomes as well. Some processes show bulbous expansions containing vesicles of different sizes, resembling the 'axon-growth cones' described in neural cultures[10]. This finding suggests that at least some of the processes are formed during culturing. Cell processes are frequently seen in contact with other cell processes or with cell somas. At the contact areas, junctional structures of the zonula adhaerentes type, formed by thickenings of adjacent plasma membranes separated by a 100–200 A gap, can be seen. Although synaptic membrane specializations are not observed, a few processes contain clusters of small (400–600 A) clear

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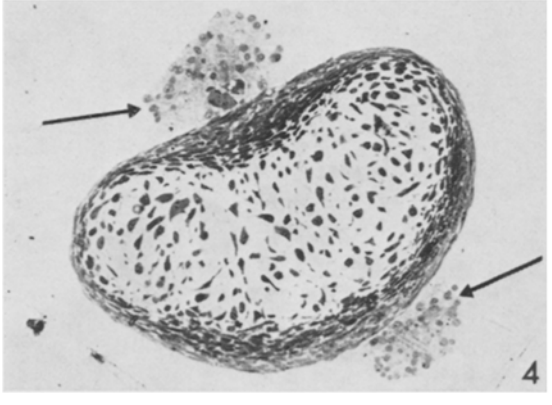

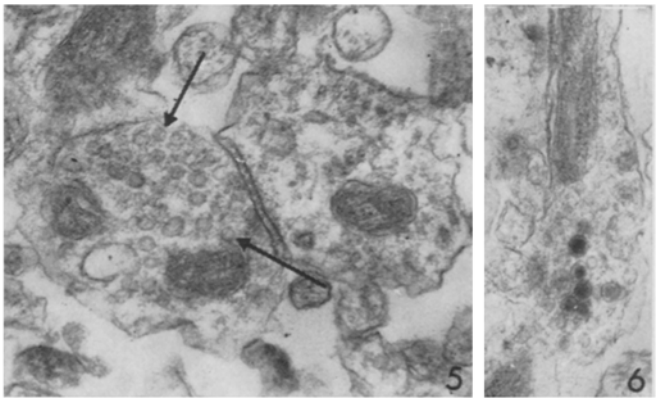

Fig. 4. Low power light micrograph of an aggregate formed by a mixture of optic lobe and limb bud cell suspensions (OL+LB) after 8 days in culture. Limb bud cells form a central core surrounded by elongated cells, whereas optic lobe cells form clusters (arrows) adherent to the latter. 1 µm Epon section, toluidine blue stained.

Fig. 5. Electron micrograph of the neural portion of the same aggregate as in Figure 4. Two cell processes appear forming a 'synaptic-like' structure. Note the numerous clear vesicles (arrows). The plasma membranes of both processes show a higher electron density at the junctional zone. Osmium tetroxide postfixation. ×30,000.

Fig. 6. Same material as in Figure 5. Another cell process shows many large vesicles with a dense central core. ×30,000.

vesicles (Figure 3), resembling the synaptic vesicles of mature synapses[11]. These vesicles seem to be best preserved in material postfixed with potassium permanganate. In some cases, isolated vesicles of 1000–1400 A in diameter, with a central dense core, can be seen along cell processes or in cell somas.

Neuroblasts found in aggregates formed by T or NR cells have the same morphological characteristics, although the latter show a peculiar spatial organization which has been described by SHEFFIELD and MOSCONA[8]. Besides occasional clusters of synaptic-like vesicles, no other synaptic elements could be observed in their processes.

'Combined' aggregates. 'Combined' OL+NR and OL+T aggregates do not show any striking ultrastructural differences when compared with 'pure' OL, NR or T ones. Although both types of 'combined' cultures apparently have a greater number of cell processes, morphological synaptic differentiation seems to be at the same stage as in 'pure' aggregates. ADLER and TEITELMAN[12] observed that, after 8 days in culture , the activity of the enzyme choline-acetyltransferase, which is responsible for the synthesis of acetylcholine, is higher in 'combined' OL+NR aggregates than in 'pure' OL or NR ones. It could seem surprising that no difference in synapse formation is found when the ultrastructure of the same aggregates is compared. On the basis that biochemical and structural aspects of differentiation must not necessarily be synchronical (see also ref.[13]), we are now studying the same type of aggregates kept in culture for longer periods.

In OL+LB 'combined' aggregates, both types of cells show a peculiar sorting-out behavior (Figure 4). After 8 days in culture, LB cells form a central core of stellated cells immersed in an amorphous extracellular substance and surrounded by a shell of elongated cells. OL cells appear forming clusters adherent to this outer shell. This tendency of reconstructed neural tissue to cover only partially other tissues in 'combined' cultures has been described by STEINBERG[14]. In the neural region of these OL+LB aggregates, there seems to be a greater number of cell processes and, moreover, synapse formation seems to be more advanced than in 'pure' OL aggregates. Many cell processes show clusters of synaptic-like vesicles of the clear type and/or larger vesicles (800–1500 A) containing a central dense core. In these 'combined' aggregates, the clusters of vesicles often appear associated with membrane specializations which suggest the existence of a synaptic complex (Figures 5 and 6).

Other authors have shown that embryonic neural cells in reaggregation cultures are able to attain functional synapse differentiation[9]. The length of time required for the appearance of synaptic structures in vitro depends on the age and type of donor tissue[3,8,13,15–17]. Seven-day-old optic lobe cells, which at this age have not formed synaptic structure in vivo[18], are able to form synapse-like structures after only 8 days in culture in combination with limb bud cells, although they fail to do so in 'pure' cultures, or in combination whit telencephalon or neural retina cells. Limb bud cells, therefore, seem to provide some factor which stimulates synapse formation by optic lobe cells[19,20].

Resumen. Se describe la diferenciación ultraestructural alcanzada en cultivos de reagregación por células neurales embrionarias despues de 8 días in vitro. Se estudiaron agregados «puros» formados por células de lóbulo óptico, retina neural o telencéfalo, y cultivos «combinados» de lóbulo óptico + retina neural (OL + NR), lóbulo óptico + telencéfalo (OL + T), y lóbulo óptico + esbozo de miembro (OL + LB). En los cultivos «puros» las células poseen características de neuroblastos, pero el proceso de sinaptogénesis está poco desarrollado. No se encuentran diferencias significativas entre la diferenciación alcanzada por los cultivos «puros» y los «combinados» OL + NR y OL + T. Por el contrario, en los «combinados» OL + LB la sinaptogénesis está mucho más avanzada y se encuentran prolongaciones sinápticas semejantes a las del adulto.

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The Allergenic Cross-Reactivity of Antisera made Against Different Molecular Weights of a Homopolymer, Dextran

Dextran, a homopolymer consisting of 96% α-1,6 linked glucose units can be obtained in fractions ranging in molecular weight from 1400 to 1.9×10^6[1]. The homopolymer may exist in preferred configurations[2]. Studies utilizing this homopolymer have demonstrated that the valency of antibody, both of the IgG and IgM class, vary with the size of the dextran[3]. Bivalent haptens can elicit the passive cutaneous anaphylaxis reaction and precipitate with antibody whereas univalent haptens cannot. RICHTER[4] has shown that a passive anaphylactic reaction (PCA) can occur if the antiserum is of sufficient strength and with dextran homopolymers of molecular weight as low as 3600. This study was undertaken to determine the degree of cross-reactivity between antibody made against various sized dextran fractions and to determine the lower limit molecular weight which will elicite an allergic reaction.

Materials and methods. New Zealand white rabbits were immunized with dextran molecular weight fractions 1400, 2950, 4875, 10,000, 110,000 and 1.9×10^6 coupled to